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In-Vivo and In-Vitro Analysis of CNS Cancers by $^1$H-NMR Spectroscopy

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Many types of neoplastic disease can effect the central nervous system, including primary cancers of the CNS such as gliomas and meningiomas as well as secondary metastatic growths from primaries in any of a number of sites. Neoplastic growth of all types may display biochemical changes different to their host tissue and alter the biochemistry of the tissue and fluid immediately surrounding the neoplasm. The use of NMR spectroscopy to detect these biochemical changes may well be of value in diagnostic medicine, particularly if those changes can be detected in-vivo by a method which is quick, safe and non-invasive. In-vitro analysis of specimens collected from cancer patients is also useful research, as this strategy is more likely is more likely to identify diagnostic markers of cancer due to the vastly superior resolution with in-vitro spectroscopy compared to in-vivo spectroscopy.

Our research goals were tested by analysing human cerebrospinal fluid (CSF) collected from patients with cancers in their CNS as well as control subjects and by analysing male Wistar rats post cell passage of the glioblastoma multiforme C6 cell line. Resected rat brain specimens were also analysed ex-vivo. Analysis by $^1$H-NMR spectroscopy was conducted using a wide bore spectrometer operating at 300MHz.

Cerebrospinal Fluid:
The cerebrospinal fluid is a clear fluid which bathes the brain and spinal cord and freely exchanges with the extracellular fluid of the CNS. Perturbations in the biochemical balance in the CNS can sometimes be detected in specimens of CSF. Specimens can be collected by puncturing the arachnoid membrane with a 22G 150mm needle passed usually between the third and fourth lumbar vertebrae of a patients spine. The fluid drips freely from the end of the needle. One hundred and eighty-four specimens of human CSF were collected for research purposes from patients at the Austin Hospital, some of these specimens coming from patients afflicted with CNS cancers. None of the specimens were collected from healthy controls so a control group of normal specimens was assembled by selecting twenty-five specimens from patients afflicted with medical conditions unlikely to effect the composition of their CSF. Specimens were lyophilised and reconstituted in deuterium oxide. Quantification of metabolites was achieved in the control and experimental specimens by comparing integral of metabolite peaks to the integral of TSP, which had been added as an exogenous reference. Comparing NMR spectra from the control group and the cancer patients revealed raised levels of lactate, alanine, valine, β-hydroxybutyrate and lowered glucose. The raised lactate and lowered glucose in the cancer patients is most likely explained by anaerobic glycolysis in the neoplastic cells, the raised alanine and valine as a product of increased cell proliferation and death, and the raised β-hydroxybutyrate possibly as a mechanism for compensating against the increased glucose consumption. There also appeared to be a correlation between the severity of the biochemical imbalance and the extent to which the cancer had advanced.

Glioblastoma multiforme in Wistar rats:
Research was conducted using forty Wistar rats. Glioblastoma multiforme cells, washed and suspended in 0.9% saline solution, were passaged in 10µL boluses into the left forebrains of twenty-two rats. Seventeen other rats underwent sham operations and one rat died perioperatively. In-vivo $^1$H NMR spectroscopy was performed regularly on the rats’ brains over a period of up to three weeks post surgery using a surface coil positioned against the rats’ craniums. The spectra obtained were not of a high enough quality to recognise distinct characteristic diagnostic markers in the spectra of the rats with GBM. However when analysing the differences between spectra
from the control and experimental groups some trends could be noted. Integrals between 1.13ppm and 1.53ppm, the region of the lactate and alanine methyl peaks, were significantly greater (p<0.05) for the rats with GBM than for the control group. In-vitro $^1$H NMR spectroscopy was conducted on ex-vivo specimens simultaneously collected from the left and right hemispheres of rats from both the control and experimental groups. Spectra from the control group were all similar and normal appearing. Spectra from the rats with GBM showed considerable variation. Raised lactate, probably from anaerobic glycolysis, and reduced N-acetylaspartate, from neuronal depletion, were common in many left forebrain spectra.

**Conclusion:**
The research demonstrated that $^1$H NMR spectroscopy could be used to demonstrate the presence of CNS cancers. Results from the study of patients and rats showed considerable variability though some clear trends did emerge. Lactate peaks, characteristic of anaerobic glycolysis, were high with CNS cancer when compared to controls in both groups and peaks characteristic of cell death were present in both groups; lowered N-acetylaspartate in the rats and raised alanine and valine in the CSF.

Results specific and definitive enough to be used to confidently make diagnostic decisions based on NMR spectra did not emerge, although the results do not negate but rather reinforce the position that NMR spectroscopy may have a more significant role in diagnostic medicine in the future.